Cobop: Microbial Biofilms: A Parameter Altering the Apparent Optical Properties of Sediments, Seagrasses and Surfaces

Alan W. Decho
Department of Environmental Health Sciences & Marine Sciences Program
University of South Carolina
Columbia, SC. 29208
Tel: (803) 777-6584. Fax: (803) 777-3391 Email: adecho@sph.sc.edu

Award #: N000149710024

LONG-TERM GOAL

The long-term goal of my research is to understand how microbial biofilms, may influence the optical properities of sediments and other surfaces in coastal oceans. The specific project goals are to determine how biofilm components may alter optical spectra through changes in reflectance, scattering and fluorescence. This project is a part of the CoBOP (Coastal Benthic Optical Properties) initiative in the Environmental Optics Program.

OBJECTIVES

The objective of year five was to pull together results collected during the second component of our field-study at Lee Stocking Island in association with the CoBOP, and to continue interactive studies with CoBOP Optics investigators. Specific objectives were to:

1) Combine data collected from sediment reflectance and scattering studies with those of biofilm studies.
2) Conduct an initial "*mass-balance*" sediment study, using both collaborative laboratory studies and existing data integration. The study will attempt to explain the reflectance properties of natural sediment.

APPROACH

Data from three sediment sites (Ooid Shoals, Twin Beaches, and North Perry) were analyzed to isolate factors that contribute to the observed alterations in sediment reflectance within the sites. This combined data from spectral reflectance studies, scattering and fluorescence work. Microbial and sedimentological properties were analyzed to characterize the intact microstructure of sediments. Laser-scanning confocal microscopy was used to characterize in-situ sediment biofilm coatings. A follow-up collaborative laboratory experiment involving Drs. Mead Allison, Rob Wheatcroft, Pamela Reid (w/ E. Louchard), Carol Stephens, and Ken Voss was run to construct a "optical sediment mass balance", involving experimental manipulations of major microbial and sedimentological properties. The effects of individual and multiple parameters on sediment reflectance profiles were determined. Data was compiled and examined for specific relationships and results were used to modify our existing model for biofilm photon trapping.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2001		**00-00-2001 to 00-00-2001**

4. TITLE AND SUBTITLE
Cobop: Microbial Biofilms: A Parameter Altering the Apparent Optical Properties of Sediments, Seagrasses and Surfaces

5a. CONTRACT NUMBER
5b. GRANT NUMBER
5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER
5e. TASK NUMBER
5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Environmental Health Sciences,& Marine Sciences Program,University of South Carolina,,Columbia,,SC, 29208

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)
11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The long-term goal of my research is to understand how microbial biofilms, may influence the optical properities of sediments and other surfaces in coastal oceans. The specific project goals are to determine how biofilm components may alter optical spectra through changes in reflectance, scattering and fluorescence. This project is a part of the CoBOP (Coastal Benthic Optical Properties) initiative in the Environmental Optics Program.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**5**	

WORK COMPLETED

All objectives were accomplished. The data collected from our year-four field campaign to Lee Stocking Island was very good. Our intensive analyses of this data allowed us to designed a "sediment mass balance" laboratory experiment involving seven different CoBOP investigators (see above). The collaborative experiment was held at RSMAS (Miami) during April 2001. Quantitative imaging, generated by nanoplast-embedded natural sediments, was conducted (at USC) using scanning confocal laser microscopy.

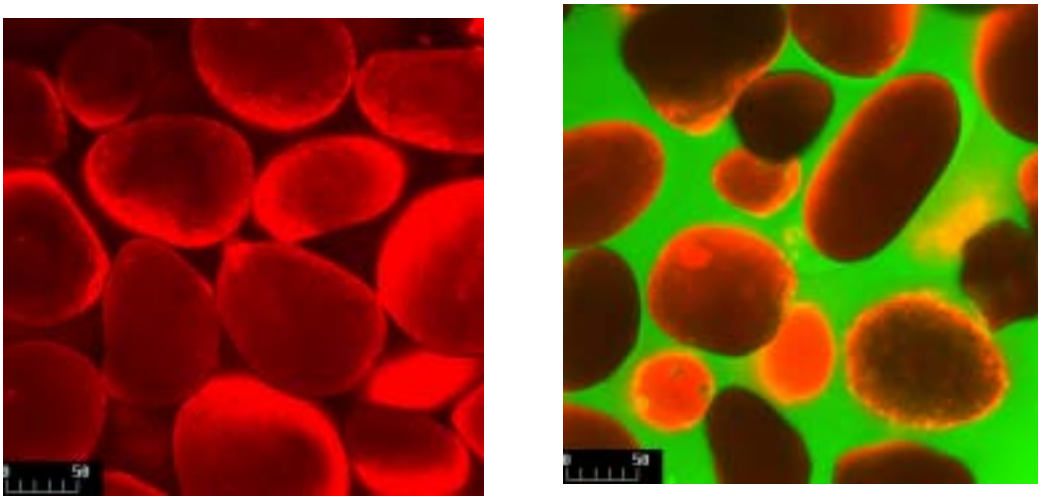
RESULTS

Previous year's results showed that biofilms, such as those in a diatom mat, caused a reduced overall spectral reflectance. This results from the exopolymer gel fraction (not the cells) of the biofilm. While exopolymers do not exert specific (wavelength-dependent absorbances), the observed reductions in reflectance may be due to enhance scattering within the exopolymer. A second major effect of a biofilm gel is the increase in fluorescence emissions (by an underlying surface) when there is an exopolymer coating on that surface. These two effects may be explained by *a "photon-trapping"* mechanism (see Fig. 1). This mechanism results from our observations of spectral absorbance, reflectance and fluoresence measurements.

We are currently refining the model into a "photon-trap" model that specifically addresses the role of translucent extracellular polymers (EPS) (secreted by microorganisms), in reducing reflectance signatures of sediments. We are compiling data from reflectance (Dr. R.P. Reid and E. Louchard), and spectral scattering measurements (w/ Dr. Ken Voss) to determine the exact mechanisms of these spectral changes resulting from the presence of Biofilms and their EPS. Our data suggest that the microbial EPS imparts a two fold effect on sediment properties. First, (1) the EPS gel alters the relative spacing of surface sediment grains (Fig. 1), allowing more light to enter (rather than reflect/scatter off of) sediments. Second, (2) the EPS gel itself effects a relevant change in the refractive index of sediment particles, and causes enhanced "forward scattering" (relative to backscatter/reflectance). Therefore, photon interacting with the surface of sediments will be "sequestered into" rather than "reflected off of" sediments due to the presence of EPS. Analyses of spectral reflectance, absorbance, scattering and changes in refractive index support now this. Our specific laboratory data have provided the controlled conditions to successfully interpret those results observed in the field.

IMPACT/APPLICATION

Critical to understanding our results, has been our ability to profile the "intact microspatial architecture" of sediments- a small-scale process that influences the larger scale optical signatures. Our work, through CoBOP, has allowed us to develop an ability to probe the hydrated microstructure of sediments using confocal- (CSLM) and multiphoton- (MP-SLM) scanning laser microscopy. Biofilms occur in varying amounts, and at virtually all sediment sites. They may exert significant alterations on the optical spectra of sediment. These alterations are detectable using a range of instrumentation. Such approaches will likely be important in understanding both optical and acoustical signatures of shallow-water sediment environments.



(A) **(B)**

Figure 1. Confocal scanning laser micrographs showing experimental sediments from "Sediment Mass Balance" experiment. (A) Micrograph showing "clean" ooid sand grains (red autofluorescence), having no biofilm coatings and associated extracellular polymers (EPS). Note close packing of sediments. This results in strong spectral reflectance profiles, smilar to those observed from natural sediment sites having no substantial biofilms. (B) Micrograph showing sediments having abundant "EPS–coatings" (green fluorescence). Such sediments exhibit a reduction in reflectance by 10 – 30 % due to spreading of sediment grains (by EPS matrix), and due to enhanced forward scattering (by EPS) that causes photons to primarily "enter" rather than be "reflected from" the sediment surface.

TRANSITIONS

The close coordination of specific "Sediment group" and "Optics group" personnel of CoBOP has provided a strong and unique dimension to our work. We are currently conducting a coordinated laboratory experiment. A second coordination involves work in conjunction with Drs. Charles Mazel and Kenneth Voss, in examining the effect of biofilm on fluorescence and reflectance signatures. This has involved both field and laboratory work. The ultimate focus of this work will be to quantitatively determine the effects of sediment-associated biofilms on alterations in Sediment Reflectance and Fluorescence profiles.

RELATED PROJECTS

None

REFERENCES

None

ONR-Sponsored Peer-Review Publications (last 5 years)

Decho, A.W. 1999. Imaging an alginate polymer gel matrix using atomic-force microscopy. *Carbohydrate Research* 315: 330-333.

Decho, A.W. and T. Kawaguchi. 1999. Confocal Imaging of in situ natual microbial communities and their extracellular polymeric secretions using Nanoplast resin. *BioTechniques* 27: 1246-1252.

Decho, A.W. 2000. Microbial biofilms in intertidal systems: an overview. *Contintental Shelf Research* 20: 1257-1273.

Kawaguchi, T. and **A.W. Decho**. 2000. Biochemical characterization of cyanobacterial extracellular polymers (EPS). *Preparative Biochemistry and BioTechnology*. 30: 321-330.

Hoffman, M. and **A.W. Decho**. 2000. Proteolytic enzymes in the marine bacterium *Pseudoalteromonas atlantica*: post-secretional activation and effects of environmental conditions. *Aquatic Microb. Ecol*. 23: 29-39.

Kawaguchi, T. and **A.W. Decho**. 2001. In situ microspatial imaging using two-photon and confocal laser scanning microscopy of bacteria and Extracellular Polymeric secretions (EPS). *Marine BioTechnology* (in press)

Submitted Manuscripts:

Decho, A.W., E. Louchard, K. Voss, M. Allison, C. Stephens, T. Kawaguchi, R.P. Reid, C.H. Mazel. Photon Trapping by the Gel Matrix of Microbial Biofilms. *Science*. (submitted)

Decho, A.W., T. Kawaguchi, M. Allison, E. Louchard, C. Stephens, R.P. Reid, K. Voss, R. Wheatcroft, B.B. Taylor. Sediment properties influencing up-welling spectral reflectance signatures: the "biofilm gel effect". *Limnol. Oceanogr*. (submitted)

Kawaguchi, T. and **A.W. Decho**. Role of the biofilm extracellular polymers in the precipitation of aragonite: a laboratory study. *Marine BioTechnology* (submitted).

Kawaguchi, T. and **A.W. Decho.** In situ analysis of carboxyl (-COOH) and sulfhydral (-SH) groups of extracellular polymeric secretions (EPS) by confocal scanning laser microscopy. *Analytical Biochemistry* (submitted)